

GENETIC PARAMETERS OF INTERSPECIFIC HYBRIDS OF *EUCALYPTUS GRANDIS* AND *E.* *UROPHYLLA* SEEDLINGS AND CUTTINGS

G. J. van den Berg^{1,3}, S. D. Verry^{2,3}, P. W. Chirwa³ and F. van Deventer¹

¹Mondi Forests (Pty) Ltd, P.O. Box 12, Hilton, 3245, South Africa.

²Creation Breeding Innovations cc, 75 Kafue Street, Lynnwood Glen, Pretoria, 0081,
South Africa

³Faculty of Natural and Agricultural Sciences, University of Pretoria,
2 Lynnwood Road, Hatfield, Pretoria, 0002, South Africa

Corresponding author : gert.vandenberg@mondigroup.co.za

ABSTRACT

The current *E. grandis* x *E. urophylla* hybrid breeding strategy of South Africa's Forestry Industry is to maintain large breeding populations of both parental species in which parents are selected based on their general combining ability (GCA) estimates or predicted individual tree breeding values and are used for interspecific hybrid crosses. The hybrid material is first screened in seedling progeny trials after which superior individuals are selected and tested as clones. Although this strategy has delivered superior clones for commercial production in South Africa, it is a time consuming strategy to follow and more cost effective strategies are being investigated. In order to review the current hybrid breeding strategy, information on the genetic control of the traits of interest is needed for *E. grandis* x *E. urophylla* seedling and clonal populations. The main objectives of this study were therefore to firstly estimate genetic parameters for *E. grandis* x *E. urophylla* hybrid seedling and clonal populations; secondly to

investigate the correlation between *E. grandis* and *E. urophylla* parental (GCA) or individual breeding values and their general hybridising ability (GHA); and lastly to determine the correlation between *E. grandis* x *E. urophylla* hybrid seedling ortets and their ramets.

Results of our study indicated that non-additive genetic variation explained the majority of the total genetic variation in *E. grandis* x *E. urophylla* seedling and clonal populations. Due to the pre-eminence of non-additive variance, the pure-hybrid correlations were weak, especially for clonal populations. It would therefore seem that GCA or predicted individual breeding values are not good predictors of GHA for growth performance in the observed populations. Our study also indicated a weak coefficient of correlation between the growth performance of seedling ortets and their ramets. These results suggest that: firstly a hybrid breeding strategy to capture non-additive genetic variation should be adopted; and secondly that the first phase of screening *E. grandis* x *E. urophylla* hybrid material as seedlings should be revisited.

Keywords

Eucalyptus grandis x *E. urophylla*; genetic parameters; seedlings and cuttings; pearson correlations.

INTRODUCTION

The deployment of interspecific hybrids in commercial forest tree planting is prevalent worldwide (Kerr et al. 2004). There are many reports on the superiority of *Eucalyptus* interspecific hybrids (Denison and Kietzka 1992; De Assis 2000; Kha and Cuong 2000; Potts et al. 2000; Verryn 2000; Vigneron et al. 2000; Potts and Dungey 2004; Bison et al. 2006). Interspecific hybrids of *E. urophylla* and *E. grandis* in particular, have been used in tropical and subtropical forestry for a while, especially in Brazil (Ikemori 1984; Bison et al. 2006), Congo (Vigneron and Bouvet 2000) and South Africa (Retief and Stanger 2009). *Eucalyptus grandis* suffers from fungal diseases, in particular, *Crysosporthe austroafricana* and *Coniothyrium* sp. cankers in these tropical and subtropical regions. However, the interspecific hybrids with *E. urophylla* have shown to be more resistant to the diseases of concern.

The current *E. grandis* x *E. urophylla* hybrid breeding strategy of South Africa's Forestry Industry is an adaption to the classic hybrid breeding strategy namely "Recurrent Selection for General Combining Ability (RS-GCA; Jenkins 1940). Large breeding populations of both parental species are maintained and elite selections are made based on either the parents GCA estimates (backward selection) or predicted individual tree breeding values (forward selection). General combining ability is a measure of the genetic worth of a parent based on

the average performance of the progeny from a particular parent, whereas predicted individual breeding values is an estimation of the genetic worth of individuals based on their own performance, and that of their sibs (Hettasch et al. 2005). Selected parents are used for interspecific hybrid crosses, and the hybrid material is first tested as seedlings in seedling progeny trials. Superior individuals are selected at mid-rotation from the seedling populations and ramets of the ortets are then tested as clones in clonal trials.

The underlying research hypothesis of the above mentioned strategy is firstly that GCA or predicted individual tree breeding values are good predictors of GHA. General hybridising ability is a measure of the genetic worth of a hybrid parent based on the average performance of the hybrid progeny from the parent when crossed with various parents of a different species (Hettasch et al. 2005). The correlation between GCA and GHA is a useful indicator of the consistency of parental performance when used as a hybrid parent compared to when the same selections are used as pure species parents. If this hypothesis is false, then a hybrid breeding strategy such as Reciprocal Recurrent Selection (RRS; Comstock et al. 1949) has obvious advantages (Vigneron and Bouvet 2000). Although some authors indicated a poor correlation between GCA and GHA (Retief and Stanger 2009; Volker et al. 2008), literature related to this topic is sparse and more information regarding this is needed. For instance, results of the latter studies were based on the performance of seedling populations and not clonal populations. In most countries, interspecific hybrids of *E. grandis* x *E. urophylla* are commercially deployed as clones. Hence genetic information on *E. grandis* x *E. urophylla* clonal populations needs to be determined.

The second underlying hypothesis of the current breeding strategy is that the performance of the seedling ortet is a good predictor of its ramets performance. Although some authors (Fuller and Little 2007; Gaspar et al. 2005; Sasse and Sands 1996) reported that there was no significant difference between eucalypt cuttings and seedlings for growth, no direct comparison of ortets and their ramets have been reported on. All of these studies produced cuttings from different individuals with a similar genetic makeup than that of the seedlings. Hence, the general theoretical literature on this subject and specifically in the context of *E. grandis* x *E. urophylla* hybrid populations is inconclusive and needs further investigation.

In addition, the magnitude of specific combining effects in the hybrid crosses (SHA) is also one of the most important factors to consider when developing a hybrid breeding strategy

(Volker 2002) and needs investigation. With this in mind, our study was set out to address the following scientific questions:

- What is the correlation between the parent's performance as a pure species (GCA or individual tree breeding values) and in a hybrid combination (GHA) in seedling and clonal populations?
- What is the magnitude of specific combining effects in the hybrid seedling and clonal populations?
- Is the performance of the seedling ortet a good predictor of the ramets performance?

Answers to these questions will provide valuable information to hybrid tree breeders around the world and will assist to construct suitable *Eucalyptus* hybrid breeding strategies. The objectives of this study were therefore to:

- Determine the Pearson correlation between *E. grandis* and *E. urophylla* parents (GCA or predicted individual tree breeding values) and their hybrid (GHA) progeny (seedlings and clones).
- Estimate genetic parameters for *E. grandis* x *E. urophylla* hybrid seedling and clonal populations.

Calculate the Pearson correlation between *E. grandis* x *E. urophylla* hybrid seedling ortets (predicted individual tree breeding values) and their ramets (mean best linear unbiased predictions [BLUP]).

MATERIALS AND METHODS

Breeding material

Seedling population

The mating design consisted of a partial factorial with 30 *E. grandis* parents (female) and 27 *E. urophylla* parents (male) (**Figure 1**). A total of 108 *E. grandis* x *E. urophylla* families were produced and established in progeny seedling trials. Although only 13% of the cells of the factorial mating were completed, 15 *E. grandis* and 15 *E. urophylla* parents were crossed between 3 and 14 times. Sixty nine of the 108 *E. grandis* x *E. urophylla* families were planted at 2 or more sites. All the parents were selected on their mature age phenotypes for growth and tree form. The *E. grandis* parents are cloned selections made in a second generation (F2) progeny trial series from the South Africa Forestry Research Institute programme (as it was called at the time). The *E. urophylla* parents used in this study are all cloned selections from an unimproved (P0) provenance/progeny trial series of open-pollinated seed collected from different provenances on Indonesian Islands.

Clonal population

A total of 148 selections were made from 63 families (**Table 1**) from the above mentioned *E. grandis* x *E. urophylla* seedling population. All trees were selected on their mature age phenotypes for growth and tree form. Multiple cuttings were produced from each selection and were established in a series of clonal trials at a minimum of 6 sites.

Table 1 Number of selections made from *E. grandis* x *E. urophylla* families and the number of clonal trials established with clonal material from each family.

Family	No. of selections	No. of clonal trials	Family	No. of selections	No. of clonal trials
G1xU12	1	6	G10xU3	2	8
G3xU17	3	6	G10xU6	2	6
G3xU19	1	6	G10xU21	1	8
G4xU3	3	6	G10xU26	1	6
G4xU6	1	6	G11xU6	1	6
G4xU8	9	6	G13xU3	1	6
G4xU12	4	6	G13xU8	2	6
G4xU16	4	6	G14xU6	1	6
G4xU18	7	6	G14xU14	2	6
G4xU19	1	6	G14xU15	2	6
G5xU17	2	6	G15xU8	5	6
G6xU1	5	6	G15xU9	1	8
G6xU8	2	6	G16xU14	7	6
G6xU9	1	8	G18xU16	1	6
G6xU13	1	8	G19xU6	1	6
G6xU14	2	6	G20xU14	3	6
G6xU16	1	6	G21xU14	2	6
G6xU17	1	6	G21xU15	2	6
G6xU18	2	6	G21xU16	1	6
G6xU27	5	6	G21xU18	2	6
G7xU5	4	6	G23xU2	4	6
G7xU8	7	6	G23xU13	1	6
G7xU10	1	8	G23xU17	2	6
G7xU13	3	6	G23xU18	1	6
G7xU18	2	8	G24xU18	1	6
G7xU19	3	6	G25xU14	4	6
G7xU20	1	6	G27xU4	1	6
G7xU21	1	8	G28xU13	1	6
G7xU22	1	8	G28xU19	5	6
G7xU23	1	8	G29xU2	1	6
G8xU13	1	6	G30xU8	1	8
G8xU17	5	6			

Trial establishment and measurements

Seedling population

Three series of trials (PE062A&B, PE80A&B&C and PE109A&B) were established in the subtropical region of South Africa, namely Zululand. Site and trial information for each trial is presented in **Table 2**. Each trial was planted in a randomised complete block design (RCB). Trial measurements were scheduled at mid-rotation (3 – 4 years) and at rotation age (7-8 years). Growth traits namely: height in metres and diameter at breast height, (DBH, in centimetres) were taken and tree volume was calculated using the following equation as described by Max and Burkhart (1976):

$$\text{Volume} = \left(\frac{\pi}{40000}\right) * k * DBH^2 * \text{Height}$$

Where,

$$k = \left(\frac{\beta_1}{3}\right) + \left(\frac{\beta_0}{2}\right) - (\beta_0 + \beta_1) + \left(\frac{\beta_2}{3}\right) * a_1^3 + \left(\frac{\beta_3}{3}\right) * a_2^3$$

$$\beta_0 = -2.72108$$

$$\beta_1 = 1.18891$$

$$\beta_2 = -0.90650$$

$$\beta_3 = 95.42845$$

$$a_1^3 = 0.83117$$

$$a_2^3 = 0.059583$$

Functions used in the equation were developed internally by Mondi Limited (Kotze and Fletcher, unpublished data).

Table 2 Site and trial information of *Eucalyptus grandis* x *E. urophylla* hybrid seedling trials.

	PE062A	PE062B	PE080A	PE080B	PE080C	PE109A	PE109B
Longitude	32° 12' E	31° 42' E	32° 11' E	31° 42' E	32° 20' E	32° 06' E	32° 06' E
Latitude	28° 34' S	29° 00' S	28° 35' S	28° 59' S	28° 16' S	28° 38' S	28° 38' S
M.A.P. (mm)	1156	1273	1271	1201	1050	1079	1079
M.A.T. (°C)	21.2	21.4	21.6	21	21.5	21.3	21.6
Altitude (m)	40	40	40	40	20	55	55
Major soil type	Fw 1110	Hu 26	Fw 1110	Hu 26	Fw 2110	Ct 2100	Ct 2100
Effective rooting depth (m)	1.51	1.51	1.51	1.51	1.51	1.51	1.51
Planting date	22/07/94	28/07/94	03/10/96	11/10/96	03/10/96	25/06/98	25/06/98
Espacement	3m x 2m	3m x 2m	3m x 2m	3m x 2m	3m x 2m	3m x 2m	3m x 2m
Fertiliser	4:1:1 @ 200g/tree	4:1:1 @ 200g/tree	4:2:0 @ 200g/tree	5:2:0 @ 70g/tree	No fertiliser	No fertiliser	No fertiliser
Replications	5	5	6	6	1	12	12
Plot size	1x6 tree line	1x6 tree line	1x6 tree line	1x6 tree line	Single tree	Single tree	Single tree
Number of families	39	36	48	34	29	33	19

Clonal population

Three *E. grandis* x *E. urophylla* clonal trial series (IC358A-F, IC361A-F and IC365A-H) were established across various sites at Zululand. Site and trial information for each trial site is presented in **Table 3**. Each trial was planted in a RCB design. Height and DBH were measured at four and seven years of age. Only four year data were available for the IC365 trial series. Tree volume was calculated using the same equation described for the seedling population.

Table 3 Site and trial information of *E. grandis* x *E. urophylla* hybrid clonal trials.

IC358 CLONAL TRIAL SERIES						
Trial	IC358A	IC358B	IC358C	IC358D	IC358E	IC358F
Longitude	32° 06' E	32° 03' E	31° 42' E	31° 43' E	31° 53' E	31° 50' E
Latitude	28° 31' S	28° 37' S	28° 59' S	28° 59' S	28° 52' S	28° 53' S
M.A.P. (mm)	1084 mm	1051 mm	1293 mm	1295 mm	1379mm	1476 mm
M.A.T. (°C)	21.6 °C	21.6°C	21.1 °C	21.1 °C	21.3°C	21.1 °C
Altitude (m)	55 m	63 m	24 m	47 m	63 m	95 m
Major soil type	Kd2000	Fw1210	Hu 27	Hu 26	Hu 2200	Hu 2200
Effective rooting depth (m)	1 – 1.4m	1.51	1.21	1.21	1.51	1.51
Planting date	2005/04/29	2005/05/09	2005/06/17	2005/08/24	2005/06/13	2005/07/05
Espacement	3m x 2m					
Fertiliser	DAP @ 60g/tree					
Replications	20	20	20	20	20	20
Plot size	Single tree					
Number of clones	68	68	68	68	68	68
IC361 CLONAL TRIAL SERIES						
Trial	IC361A	IC361B	IC361C	IC361D	IC361E	IC361F
Longitude	32° 12' E	32° 08' E	31° 58' E	31° 49' E	31° 41' E	31° 42' E
Latitude	28° 36' S	28° 39' S	28° 44' S	28° 54' S	29° 00' S	28° 59' S
M.A.P. (mm)	1201	1116	1198	1486	1273	1259
M.A.T. (°C)	21.6	21.5	21.3	21.1	21	21.1
Altitude (m)	55	71	76	79	63	47
Major soil type	Fw1110	Vf 2110	Hu 26	Hu 2200	Hu 26	Fw32
Effective rooting depth (m)	1.51	1.51	1.21	1.51	1.51	0.5 – 0.8
Planting date	2006/08/23	2006/09/27	2006/09/15	2006/10/26	2006/08/17	2006/08/18
Espacement	3m x 2m					
Fertiliser	DAP @ 60g/tree					
Replications	20	20	20	20	20	20

Statistical analysis

Descriptive statistics

In order to compute the descriptive statistics of each trial site for all the growth traits, PROC MEANS in SAS (SAS Institute 2002) was used. The descriptive analysis was done prior to the standardisation of the data.

Standardisation of data

Forest trees often display heterogeneous variances for growth traits where a strong relationship between the mean of the trait and its phenotypic and genetic variances are seen. This relationship is such that the field trials with bigger trees will have larger phenotypic and genetic variances than the field trials with smaller trees even if the trials are at the same age (Hodge and Dvorak, 2012). In order to deal with these differences in scale, White et al. (2007) recommend data standardisation prior to analysis of variances, variance component analysis, or multi-site mixed model analysis. The standardisation of data homogenised variances that were used together in the linear model and eliminated any spurious genotype x environment interaction (Burdon 1977; Eisen and Saxon 1983; Hill 1984). The standardisation for the analysis of this paper was performed as described by Hodge and Dvorak (2012). First, the coefficient of variation (CV) was calculated for each growth trait for each replication at each site. The mean coefficient of variances (CV_y) for each family-site-trait combination was also calculated. The phenotypic observations were then standardised with PROC STANDARD in SAS (SAS Institute 2002) for each replication at each site to a mean = 100, and with a standard deviation of = $100 \times CV_y$. This is equal to dividing all observations by the phenotypic standard deviation (SD), as recommended by White et al. (2007), followed by adding a constant (100%) and multiplying by a constant ($100 \times CV_y$). The population mean for the growth trait can therefore be interpreted as 100%, and the associated variances and SD are the same size relative to mean as in the raw data. Predicted breeding values and all variance components can thus be directly interpreted as percentage gain (above or below 100%) without back-transformation or rescaling.

Analysis of the E. grandis x E. urophylla seedling population

The statistical model used for the factorial was as follows:

$$y_{ijklm} = \mu + S_i + R_{j(i)} + f_k + m_l + fm_{kl} + S_i*fm_{ikl} + e_{ijklm}$$

Where,

y_{ijklm} = the m^{th} observation of the j^{th} replication for the kl^{th} family at the i^{th} site;

μ = overall mean;

S_i = fixed effect of the i^{th} site;

$R_{j(i)}$ = fixed effect of the j^{th} replication within the i^{th} site;

f_k or m_l = the random GHA effect for the k^{th} female or the l^{th} male;

fm_{kl} = random specific hybridising ability (SHA) effect of the k^{th} and l^{th} parents;

S_i*fm_{jkl} = random SHA by Site Interaction;

e_{ijklm} = random within plot error term;

All effects, except the overall mean, site and replication effect, were assumed to be random and independently distributed. A mixed model using PROC MIXED (SAS Institute 2002) in SAS was used to estimate variance components and to obtain BLUP estimates of random genetic effects (GHA and SHA) simultaneously. Restricted maximum likelihood (REML) was used in order to maximise the likelihood of the sample residuals. Wald test was used to determine the significance of the random effects.

The relationship between variance components derived from the quantitative genetic model was used to estimate the additive and dominance variance (Falconer 1981).

$\hat{\sigma}_f^2$ = variance due to the female effect,

$\hat{\sigma}_{Af}^2 = 4\hat{\sigma}_f^2$ is the additive variance due to the female effect,

$\hat{\sigma}_m^2$ = variance due to the male effect,

$\hat{\sigma}_{Am}^2 = 4\hat{\sigma}_m^2$ is the additive variance due to the male effect,

$\hat{\sigma}_A^2 = 1/2(\hat{\sigma}_{Af}^2 + \hat{\sigma}_{Am}^2)$ is the additive variance combining the female and male effect,

$\hat{\sigma}_{fm}^2$ = variance due to the family effect,

$\hat{\sigma}_D^2 = 4\hat{\sigma}_{fm}^2$ is the dominance variance,

$\hat{\sigma}_G^2 = \hat{\sigma}_A^2 + \hat{\sigma}_D^2$ is the total genetic variance,

$\hat{\sigma}_{total}^2 = \hat{\sigma}_G^2 + \hat{\sigma}_e^2$ is the total phenotypic variance.

Heritabilities were estimated as:

$\frac{2}{f} = \frac{\hat{\sigma}_{Af}^2}{\hat{\sigma}_{total}^2}$ is the narrow-sense heritability for the female half-sibs,

$\frac{2}{m} = \frac{\hat{\sigma}_{Am}^2}{\hat{\sigma}_{total}^2}$ is the narrow-sense heritability for the male half-sibs,

$\frac{2}{i} = \frac{\hat{\sigma}_A^2}{\hat{\sigma}_{total}^2}$ is the narrow-sense heritability for the combined female and male hybrid parents,

$d^2 = \frac{\hat{\sigma}_D^2}{\hat{\sigma}_{total}^2}$ is the ratio of dominance variance to total individual phenotypic variance,

$H_i^2 = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_{total}^2}$ is the broad-sense heritability on an individual basis,

Standard errors of heritabilities were calculated by Dickerson's approximation (Dickerson 1969).

In order to determine the genetic correlations of the same trait expressed on multiple sites as described by Burdon (1977), type B genetic correlations were estimated as:

$r_{Bfm} = \frac{\hat{\sigma}_{fm}^2}{\hat{\sigma}_{fm}^2 + \hat{\sigma}_{s*fm}^2}$ is the type B genetic correlation for the full-sib families.

Where,

$\hat{\sigma}_{fm}^2$ = variance due to the family effect;

$\hat{\sigma}_{s*fm}^2$ = variance due to the family by site interaction effect.

Type B correlation estimates the magnitude of genotype by environment interaction (GxE) that is due to rank changes across environments. This correlation over multiple sites can range between zero and one. An $r_B = 1$ predicts a perfect correlation between performance in different environments. In tree breeding, the generally accepted type B correlation estimate is equal to 0.7 (Hettasch et al. 2005).

The Pearson correlation coefficient between the GCA of *E. grandis* parents and the GHA of their interspecific hybrid progeny was estimated by using PROC CORR (SAS Institute 2002) in SAS. In the case of *E. urophylla* parents however, intraspecific progeny were not available to calculate the GCA values of the *E. urophylla* parents. Instead, individual tree breeding values were used for estimating the above mentioned correlation coefficient. The individual tree breeding values of the *E. urophylla* pure species parents were estimated using BLUP analysis (Van den Berg et al. 2016a).

Analysis of the E. grandis x E. urophylla clonal population

The statistical model used for the factorial was as follows:

$$y_{ijklmn} = \mu + S_i + R_{j(i)} + f_k + m_l + fm_{kl} + c_m + S_i * fm_{ikl} + S_i * c_{im} + e_{ijklmn}$$

Where,

y_{ijklmn}	= the n^{th} observation of the j^{th} replication for the kl^{th} family for the m^{th} clone at the i^{th} site;
μ	= overall mean;
S_i	= fixed effect of the i^{th} site;
$R_{j(i)}$	= fixed effect of the j^{th} replication within the i^{th} site;
f_k or m_l	= the random GHA effect for the k^{th} female or the l^{th} male;
fm_{kl}	= random SHA effect of the k^{th} and l^{th} parents;
C_m	= random effect of the m^{th} Clone;
$S_i * fm_{jkl}$	= random SHA by Site Interaction;
$S_i * c_m$	= random Clone by Site Interaction;
e_{ijklm}	= random within plot error term;

All effects, except the overall mean, site and replication effect, were assumed to be random and independently distributed. As with the seedling population, PROC MIXED (SAS Institute 2002) in SAS was used to estimate variance components and to obtain BLUP of random genetic effects (GHA, SHA and clone) simultaneously. Additive genetic variance, dominance genetic variance and heritabilities were calculated the same way as for the hybrid seedling population with the following exceptions:

$$\hat{\sigma}_{NA}^2 = \hat{\sigma}_D^2 + \hat{\sigma}_C^2 \text{ is the total non-additive genetic variance,}$$

$$\hat{\sigma}_G^2 = \hat{\sigma}_A^2 + \hat{\sigma}_{NA}^2 \text{ is the total genetic variance,}$$

The ratio of clone variance to total individual phenotypic variance was estimated as:

$$c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_{total}^2}$$

The type B genetic correlation for the clone effect was estimated as:

$$r_{Bc} = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_c^2 + \hat{\sigma}_{s*c}^2}$$

Where,

$$\hat{\sigma}_c^2 \text{ = variance due to the clone effect;}$$

$$\hat{\sigma}_{s*c}^2 \text{ = variance due to the clone by site interaction effect.}$$

PROC CORR (SAS Institute 2002) in SAS was used to estimate the Pearson correlation coefficients for volume between selected *E. grandis* x *E. urophylla* ortets and their ramets.

RESULTS

Descriptive statistics of *Eucalyptus grandis* x *E. urophylla* seedling and clonal trials

Descriptive statistics for DBH, height, tree volume and survival of the *E. grandis* x *E. urophylla* seedling progeny trials and clonal trials are presented in **Table 4** and **Table 5** respectively. The survival rates ranged between 79.4% and 92.3% for the seedling progeny trials and between 75.7% and 95.5% for the clonal trials. The mean DBH (age=7 years) for the seedling and clonal trials with the best growth rates was 24.5 cm (SD=4.65) and 17.38 (SD=3.7) respectively. The lowest mean DBH was 17.6 cm (SD=5.61) for the seedling progeny trials and 12.4 cm (SD=2.22) for the clonal trials. Overall, the standard deviations were higher for the seedling progeny trials (between 4.65 and 5.61) than for the clonal trials (between 2.22 and 3.82) for DBH at 7 years of age. A similar pattern was seen for height and tree volume.

Table 4 Means and ranges from the *E. grandis* x *E. urophylla* seedling progeny trials for diameter at breast height (DBH in cm), height (m) and tree volume (m³).

Site	N	Age	DBH	SD	Range	Height	SD	Range	Volume	SD	Range	Survival	SD
PE062A	1290	7	19.9	5.07	5.7-33.5	25.1	6.26	9.7-36.9	0.369	0.247	0.011-1.273	80.8	39.42
PE062B	1080	7	24.5	4.65	9.2-36.4	20.9	5.29	9.7-34.0	0.44	0.231	0.029-1.181	86.5	34.21
PE080A	1728	4	14.6	2.51	5.3-20.4	17.1	2.03	7.3-20.9	0.121	0.044	0.007-0.260	92.3	26.61
PE080B	1224	4	13.6	2.05	6.2-18.9	13.9	1.23	6.6-18.1	0.085	0.028	0.009-0.184	79.4	40.45
PE080C	894	4	11.2	2.05	5.2-16.5	13.2	1.73	5.8-18.1	0.056	0.023	0.005-0.128	88.8	31.51
PE109A	396	7	17.6	5.61	7.1-29.9	24.1	6.03	10.1-35.6	0.293	0.216	0.016-0.904	88.9	31.41
PE109B	228	7	17.9	5.59	7.5-29.2	23.2	5.34	10.5-32.7	0.288	0.211	0.018-0.842	88.2	32.42

Table 5 Means and ranges of the *E. grandis* x *E. urophylla* clonal trials for diameter at breast height (DBH in cm), height (m), tree volume (m³) and survival (%).

Site	N	Age	DBH	SD	Range	Height	SD	Range	Volume	SD	Range	Survival	SD
IC358AKWA	1228	4	13.83	2.62	6.1-21.3	18.67	2.08	8.68-22.5	0.12	0.05	0.011-0.314	90.29	29.61
	1222	7	15.54	3.57	6.2-27.1							89.85	30.21
IC358BKWA	1224	4	13.19	2.20	5.6-19.2	17.26	1.81	8.12-21.9	0.10	0.04	0.009-0.236	90.22	29.71
	1196	7	15.00	3.17	5.8-25							87.94	32.58
IC358CMTZ	1233	4	12.19	1.66	6-16.8	14.64	1.16	8.4-17.5	0.07	0.02	0.009-0.151	90.66	29.11
	1173	7	13.99	2.48	6-20.5							86.25	34.45
IC358DMTZ	1114	4	12.56	2.11	5.5-19.5	15.26	1.48	7.7-19.9	0.08	0.03	0.007-0.238	81.91	38.51
	1030	7	14.68	3.20	5.5-24.2							75.74	42.88
IC358EPDF	1078	4	13.85	3.00	5.1-20.7	16.46	2.20	9-21.7	0.11	0.05	0.008-0.285	79.26	40.56
	1032	7	15.35	3.63	6.3-25.4							75.88	42.80
IC358FPDF	1090	4	13.72	2.84	5.1-20.8	15.93	2.12	8.26-21.4	0.10	0.05	0.008-0.286	80.15	39.90
	1046	7	15.89	3.82	5.9-26.7							76.91	42.16
IC361AKWA	1605	4	14.05	2.51	6-20.6	18.78	2.09	9.8-22.7	0.12	0.05	0.012-0.295	94.47	22.86
	1601	7	15.92	3.69	6.2-27.1							94.18	23.43
IC361BKWA	1623	4	14.96	2.60	5.5-21.5	19.30	1.99	11-22.9	0.14	0.06	0.01-0.332	95.47	20.80

	1602	7	16.98	3.73	5.5-27							94.20	23.38
IC361CPDF	1592	4	10.33	1.48	5.4-15.4	11.93	0.88	8-15.2	0.04	0.01	0.009-0.109	93.65	24.40
	1537	7	12.44	2.22	5.6-19.9							90.36	29.53
IC361DPDF	1351	4	14.87	2.59	5.3-21.4	17.31	1.69	9-21.9	0.13	0.05	0.009-0.29	79.41	40.45
	1338	7	17.38	3.72	5.3-27							78.71	40.95
IC361EMTZ	1575	4	12.37	2.30	5.6-20.5	15.72	1.52	9.2-19.6	0.08	0.03	0.01-0.259	92.71	26.01
	1528	7	14.99	3.57	5.6-28							89.88	30.17
IC361FMTZ	1609	4	12.51	2.27	5.3-18.5	16.29	1.97	7.8-20.1	0.09	0.04	0.008-0.215	94.65	22.52
	1532	7	14.46	3.22	5.5-22.6							90.06	29.93
IC365ATEZ	439	4	10.75	1.96	5.4-15.9	14.48	1.61	10.1-18.7	0.06	0.02	0.009-0.149	91.46	27.98
IC365BFCN	452	4	12.67	2.53	5.5-19	16.07	2.09	10.2-21.3	0.09	0.04	0.009-0.242	94.17	23.46
IC365CFNW	442	4	11.20	2.04	5.9-16.5	14.85	1.68	10.5-19.2	0.06	0.03	0.012-0.165	92.05	27.08
IC365DPDF	393	4	16.06	3.64	6.1-24.7	18.86	3.00	10.7-25.9	0.17	0.09	0.012-0.499	81.88	38.56
IC365EMTZ	401	4	13.45	2.46	5.2-19.1	16.71	2.02	9.9-21.4	0.10	0.04	0.008-0.246	83.54	37.12
IC365FPDF	419	4	15.65	2.86	5.8-23.3	18.52	2.36	10.4-24.8	0.15	0.07	0.011-0.424	87.29	33.34
IC365GKWA	407	4	15.26	3.24	5.7-23.2	18.20	2.67	10.3-24.7	0.15	0.07	0.011-0.419	84.79	35.95
IC365HDUK	438	4	11.74	2.35	5.6-18.3	15.30	1.93	10.2-20.7	0.07	0.03	0.01-0.218	91.25	28.29

Variance component estimates and genetic parameters of *Eucalyptus grandis* x *E. urophylla* seedling and clonal populations

Variance component estimates and genetic parameters are presented in **Tables 6** and **7** for the *E. grandis* x *E. urophylla* seedling population, and in **Tables 8** and **9** for the clonal population. The female variance component estimates ($\hat{\sigma}_f^2$) for the *E. grandis* hybrid parents were insignificant for the seedling ($p < 0.409$ for DBH) and clonal ($p < 0.188$ for DBH) populations (**Tables 6** and **8**). The estimates of the female variance components ranged between zero (height and volume) and 0.76 (DBH) for the seedling population, and between 0.43 (height) and 26.55 (volume) for the clonal population. Consequently, the additive genetic variance and narrow-sense heritability were also low for the *E. grandis* hybrid parents in the seedling population ($\hat{\sigma}_{Af}^2 = 3.04$ and $h_f^2 = 0.005$ for DBH) and the clonal population ($\hat{\sigma}_{Af}^2 = 15.12$ and $h_f^2 = 0.05$ for DBH) as presented in **Tables 7** and **9**.

The GCA estimates of the same *E. grandis* parents used in this study were between -9% and 12% and narrow-sense heritability 0.24 for volume in a full-sib pure species population (Van den Berg et al. 2016). This is an indication that selecting for additive gene effects based on the *E. grandis* pure species parent performance may not necessary lead to genetic gains in growth if the same *E. grandis* parents are used as hybrid partners with *E. urophylla*. However, it must be born in mind that the *E. grandis* parents were selected from a second generation improved population and could allow for a more homogeneous population than the *E. urophylla* parents. The genetic contribution to growth from the *E. urophylla* hybrid parents was significant ($p < 0.05$ for volume) for the seedling population, but not for the clonal population (**Tables 6** and **5.8**). Narrow-sense heritability for the *E. urophylla* hybrid parents in the seedling population was 0.24 for tree volume (**Table 7**) and 0.07 in the clonal population (**Table 9**). On average however, additive genetic variance ($\hat{\sigma}_A^2 = 388.48$) only explained 32% of the total genetic variation ($\hat{\sigma}_G^2 = 1212.34$) in the seedling population and narrow-sense heritability using the combined female and male additive effect was estimated at 0.12 for volume (**Table 7**). In the case of the clonal population, additive genetic variation ($\hat{\sigma}_A^2 = 109.56$) only explained 29% of the total genetic variance ($\hat{\sigma}_G^2 = 379.40$) for volume at four years (**Table 9**). However, when the additive genetic variance was investigated in the clonal population, the progeny of *E. grandis* and *E. urophylla* hybrid parents performed similar with narrow-sense heritabilities estimated at 0.06 and 0.07 respectively for volume at four years.

A potential reason for the relatively low overall additive genetic variation could be due to the reduced genetic base of the parent populations. Parents were selected for growth, good form and resistance to parasites. This selection process allowed for a more homogeneous population for growth traits of the *E. grandis* and *E. urophylla* parents.

The genetic control of full-sib *E. grandis* x *E. urophylla* families was highly significant ($p < 0.001$) for all growth traits in the seedling population (**Table 6**). The ratio of dominance variance to total variance (d^2) were estimated at 0.20 for DBH, 0.24 for height and 0.25 for tree volume (**Table 7**). Dominance variance ($\hat{\sigma}_D^2 = 823.86$) accounted for 68% of the total genetic variance ($\hat{\sigma}_G^2 = 1212.34$) for volume. The full-sib hybrid family growth performance in the seedling population was relatively stable across sites and little GxE was detected ($r_{Bfm} = 0.74$ for DBH, 0.75 for height and 0.84 for volume).

In the case of *E. grandis* x *E. urophylla* clonal population, non-additive genetic variation ($\hat{\sigma}_{NA}^2 = 269.84$) explained 71% of the total genetic variation ($\hat{\sigma}_G^2 = 379.40$) of the four year volume data (**Table 9**). Only 21.6% of the non-additive variation ($\hat{\sigma}_{NA}^2 = 269.84$) could be explained by the dominance variance ($\hat{\sigma}_D^2 = 58.39$) and the full-sib hybrid family effect was insignificant as indicated in **Table 8**. The rest of the non-additive genetic variation was explained by the clone effect ($\hat{\sigma}_C^2 = 211.46$ for volume) and was highly significant ($p < 0.001$) for the three growth traits (**Tables 8 and 9**). However, it must be borne in mind that, in many cases, only one or two individuals were selected from a specific cross to test as clones. Hence, the data from this clonal population might be inadequate to partition the non-additive genetic variance into dominance and epistatic genetic variance. In order to try and shed some light onto the nature of non-additive and additive genetic effects, the random clonal effect was dropped from the linear model. It is apparent from **Table 9** that when clone effects were dropped from the model, the ratio explained by the dominance variance increased substantially from 0.04 to 0.27 for volume. The narrow-sense heritabilities for the *E. grandis* and the *E. urophylla* hybrid parents stayed low at 0.07 each. This is an indication that effects which the model previously allocated to remaining genetic effects among clones within a family are now mostly being absorbed by the inferred dominance genetic component of variation. This result points to a strong confounding effect between dominance and other epistatic terms present in the clone effect. Nevertheless, results from both the *E. grandis* x *E. urophylla* seedling and hybrid population indicated that a breeding strategy to capture non-additive genetic effects will be the most appropriate strategy to follow.

Table 6 Variance components of the random effects of the *E. grandis* x *E. urophylla* hybrid seedling population for diameter at breast height (DBH), height and tree volume.

	Effect	Estimate	SE	ZValue	ProbZ
DBH	female	0.76	3.29	0.23	0.409
	male	32.61	21.07	1.55	0.061
	full-sib hybrid families	30.93	9.75	3.17	0.001
	site*full-sib hybrid families	10.72	4.73	2.27	0.012
	error	533.64	11.15	47.86	<0.001
Height	female	0			
	male	16.56	10.86	1.52	0.064
	full-sib hybrid families	21.75	5.89	3.69	<0.001
	site*full-sib hybrid families	7.06	2.94	2.40	0.008
	error	312.53	6.53	47.87	<0.001
Volume	female	5.32E-17			
	male	194.24	114.46	1.70	0.045
	full-sib hybrid families	205.97	54.90	3.75	<0.001
	site*full-sib hybrid families	40.32	22.63	1.78	0.037
	error	2797.50	58.46	47.86	<0.001

Table 7 Genetic parameters of the *E. grandis* x *E. urophylla* hybrid seedling population for diameter at breast height (DBH), height and tree volume. $\hat{\sigma}_{Af}^2$ or $\hat{\sigma}_{Am}^2$ = additive genetic variance due to the female or male effect, h_f^2 or h_m^2 = narrow-sense heritability for female or male half-sibs, $\hat{\sigma}_D^2$ = dominance genetic variance, d^2 = ration of dominance variance to total phenotypic variance, r_{Bfm} = type B correlation for the full-sib families x site interaction $\hat{\sigma}_A^2$ = additive genetic variance combining the female and male effect, h_i^2 = narrow-sense heritability for the combined female and male effects, $\hat{\sigma}_G^2$ = total genetic variance and H_i^2 = broad-sense heritability.

female (<i>E. grandis</i>)	DBH	Height	Volume
$\hat{\sigma}_{Af}^2$	3.04±13.27	0	0
h_f^2	0.005±0.02	0	0
male (<i>E. urophylla</i>)			
$\hat{\sigma}_{Am}^2$	130.46±84.29	66.22±43.45	776.97±457.83
h_m^2	0.21±0.14	0.19±0.12	0.24±0.14
full-sib hybrid families			
$\hat{\sigma}_D^2$	123.71±38.98	86.98±23.67	823.86±219.59
d^2	0.20±0.06	0.24±0.07	0.25±0.07
r_{Bfm}	0.74	0.75	0.84
$\hat{\sigma}_A^2$	66.75	33.11	388.48
h_i^2	0.11	0.09	0.12
$\hat{\sigma}_G^2$	190.45	120.09	1212.34
H_i^2	0.31	0.34	0.37
$\hat{\sigma}_{total}^2$	608.67	357.89	3238.03

Table 8 Variance components of the random effects of the *E. grandis* x *E. urophylla* hybrid clonal population for diameter at breast height (DBH), height and tree volume.

	Effect	Estimate	StdErr	ZValue	ProbZ
DBH	female	3.78	4.27	0.89	0.188
	male	5.42	4.37	1.24	0.108
	full-sib hybrid families	2.13	5.57	0.38	0.351
	clone	36.33	5.89	6.16	<0.001
	site*full-sib hybrid families	1.32	1.37	0.96	0.167
	site*clone	18.92	2.16	8.76	<0.001
	error	251.49	2.94	85.46	<0.001
Height	female	0.43	1.28	0.34	0.367
	male	0.83	1.25	0.66	0.254
	full-sib hybrid families	2.43	2.05	1.18	0.118
	clone	11.87	1.94	6.13	<0.001
	site*full-sib hybrid families	0.36	0.54	0.68	0.250
	site*clone	5.76	0.78	7.37	<0.001
	error	97.91	1.15	85.44	<0.001
Volume	female	26.55	27.62	0.96	0.168
	male	28.23	23.70	1.19	0.117
	full-sib hybrid families	14.60	31.56	0.46	0.322

clone	211.46	33.71	6.27	<0.001
site*full-sib hybrid families	4.57	7.02	0.65	0.257
site*clone	103.13	11.38	9.07	<0.001
error	1283.82	15.02	85.48	<0.001

Table 9 Genetic parameters of the *E. grandis* x *E. urophylla* hybrid clonal population for diameter at breast height (DBH), height and tree volume. $\hat{\sigma}_{Af}^2$ or $\hat{\sigma}_{Am}^2$ = additive genetic variance due to the female or male effect, h_f^2 or h_m^2 = narrow-sense heritability for female or male half-sibs, $\hat{\sigma}_D^2$ = dominance genetic variance, d^2 = ration of dominance variance to total phenotypic variance, r_{Bfm} = type B correlation for the full-sib families x site interaction, c^2 = ration of clone variance to total phenotypic variance, r_{Bc} = type B correlation for the clone x site interaction, $\hat{\sigma}_A^2$ = additive genetic variance combining the female and male effect, h_i^2 = narrow-sense heritability for the combined female and male effects, $\hat{\sigma}_G^2$ = total genetic variance and H_i^2 = broad-sense heritability.

female (<i>E. grandis</i>)	With clone			Without clone		
	DBH	Height	Volume	DBH	Height	Volume
$\hat{\sigma}_{Af}^2$	15.12±17.08	1.73±5.12	106.20±110.48	15.99±16.74	3.65±5.12	117.56±108.26
h_f^2	0.05±0.05	0.01±0.04	0.06±0.07	0.05±0.05	0.03±0.04	0.07±0.06
male (<i>E. urophylla</i>)						
$\hat{\sigma}_{Am}^2$	21.66±17.48	3.32±5.00	112.91±94.82	20.86±16.56	3.75±4.63	118.85±93.97
h_m^2	0.07±0.06	0.03±0.04	0.07±0.06	0.064±0.05	0.03±0.038	0.07±0.05
full-sib hybrid families						
$\hat{\sigma}_D^2$	8.51±22.28	9.72±8.21	58.39±126.24	79.08±23.62	28.07±8.21	452.74±135.17
d^2	0.03±0.07	0.08±0.07	0.04±0.08	0.24±0.07	0.23±0.068	0.27±0.08
r_{Bfm}	0.62	0.87	0.76	0.58	0.58	0.59
clone						
c^2	0.12±0.07	0.1±0.07	0.13±0.08			
r_{Bc}	0.66	0.67	0.67			
$\hat{\sigma}_A^2$	18.39	2.53	109.56	26.42	3.7	118.21
$\hat{\sigma}_{NA}^2$	44.84	21.59	269.84	79.08	28.07	452.74
$\hat{\sigma}_G^2$	63.24	24.12	379.40	105.5	31.77	570.95
H_i^2	0.20	0.20	0.23			
$\hat{\sigma}_{total}^2$	314.78	118.96	1644.96	324.59	121.05	1703.94

Correlation between pure species parents and hybrid parents growth performance

The above results indicated that when selecting for additive gene effects, *E. urophylla* hybrid parents played a bigger role than the *E. grandis* hybrid parents when the growth performance of *E. grandis* x *E. urophylla* hybrid seedlings was compared. The success of the current hybrid breeding strategy will however still depend on the correlation between the breeding values of the selected *E. urophylla* pure species parents and the GHA estimates of the *E. urophylla* hybrid parents.

In our study, the Pearson correlation between predicted individual breeding values of the *E. urophylla* pure species parents and the GHA of the *E. urophylla* hybrid parents was estimated at 0.58 ($p < 0.007$) as indicated in **Table 10**. The individual breeding values of the selected *E. urophylla* pure species parents ranged between -3.5% to 21.6% gain (Van den Berg et al. 2016a). However, the correlation between family breeding values of the *E. urophylla* pure species families and the GHA estimates of the *E. urophylla* hybrid parents were lower (0.02, $p < 0.943$), even though the range of the *E. urophylla* family breeding values (-7.8% to 19.7%) were similar to that of the individual breeding values as estimated by Van den Berg et al. (2016a).

Table 10 Pearson correlation coefficients between *E. urophylla* pure species parent family and individual breeding values (BV) and general hybridizing ability (GHA) of the *E. urophylla* hybrid parents. Prob > |r| under H0: Rho = 0.

Variable	N	Mean	Range	Correlation with GHA	p-value
GHA	20	1.89±9.45	-17.52 - 16.08	1	
Family BV	20	5.30±8.84	-7.75 - 19.69	0.017	0.943
Individual BV	20	12.20±5.74	-3.45 - 21.60	0.581	0.007

This result suggests that individual breeding values of the *E. urophylla* pure species are relatively good indicators of *E. urophylla* parent performance as hybrid partners with *E. grandis* if tree volume is the trait of interest. Hence, the selection for additive gene effects of *E. urophylla* pure species parents will lead to genetic gains in growth traits of *E. grandis* x *E. urophylla* hybrid seedlings derived from the selected *E. urophylla* parents. However, these results are based on the performance of the *E. grandis* x *E. urophylla* hybrid seedling population. In most countries, interspecific hybrids of *E. grandis* x *E. urophylla* are commercially deployed as clones, and an important factor that should be kept in mind when

deciding on the best *E. grandis* x *E. urophylla* hybrid breeding strategy is the correlation between the selected ortet and the ramets of the ortet.

Correlation between the standardised volumes and BLUPs of *E. grandis* x *E. urophylla* ortet and ramets

The individual tree volume of 126 *E. grandis* x *E. urophylla* seedling ortets and the mean tree volume of their ramets were used to estimate the phenotypic correlation between ortets and their ramets (**Table 11**). Standardised data were used to estimate the Pearson correlation coefficients. The percentage gain in individual tree volume of the selected seedling ortets that were used in calculating the correlation coefficients ranged between 1% and 197% over the *E. grandis* x *E. urophylla* hybrid seedling population mean. The tree volume means of their ramets ranged between -46% and 54% over the clonal population mean. The phenotypic correlation between the individual tree volumes of the ortets and the mean tree volumes of their ramets was positive and estimated at 0.3174 ($p < 0.0003$).

In addition to the phenotypic correlations, the family and individual breeding values of the *E. grandis* x *E. urophylla* seedling ortets were correlated (Pearson) to the mean clonal BLUP estimates of their ramets. Clonal individual and family breeding values could not be calculated due to the insignificance ($p < 0.322$) of the full-sib hybrid family effect in the clonal population. For the same reason, a reliable genetic correlation between *E. grandis* x *E. urophylla* hybrid ortets and ramets could not be calculated.

The family breeding values of the seedling population ranged between -25% and 39.5%, and between -8% and 61% for the individual breeding values (**Table 11**). The mean clonal BLUP estimates for the clonal population ranged between -31% and 38%. A correlation coefficient of 0.1522 ($p < 0.0901$) was detected between the ortet family breeding values and the mean BLUP estimates of their ramets. In the case of individual breeding values, a higher correlation coefficient of 0.2481 ($p < 0.0053$) was estimated between the ortet individual breeding values and the mean BLUP estimates of the clones. The best correlation coefficient (0.3355, $p < 0.0001$) however, was detected between the individual breeding values of the ortets and the mean tree volumes of their ramets.

In general, the correlation between seedling ortets and their ramets was relatively weak for all the variables used and is an indicator that the best seedling does not necessarily produce the best clone. The feasibility of selection at seedling level therefore needs to be investigated.

Table 11 Pearson correlation coefficients between *E. grandis* x *E. urophylla* seedling ortets and their ramets for tree volume. BV = breeding values, std = standardized data, blup = best linear unbiased prediction estimates. N=126, Prob > |r| under H0: Rho = 0.

		Clonal ramets	
		mean std volume (Range: -46% - 54%)	clonal blups (Range: -31% - 38%)
Seedling ortets	individual std volume (Range: 1% - 197%)	0.3174 ($p < 0.0003$)	0.2673 ($p < 0.0026$)
	family bv (Range: -25% - 39.5%)	0.22566 ($p < 0.0114$)	0.1522 ($p < 0.0901$)
	individual bv (Range: -8% - 61%)	0.3355 ($p < 0.0001$)	0.2481 ($p < 0.0053$)

DISCUSSION

The results from our study indicated that non-additive genetic variation explained majority of the genetic variation present in *E. grandis* x *E. urophylla* seedling and clonal populations. The same phenomenon was found by other authors for *E. grandis* x *E. urophylla* hybrid seedlings (Bouvet et al. 2009; Retief and Stanger 2009; Rezende and de Resende 2000; Vigneron et al. 2000). Retief and Stanger (2009) reported that dominance genetic effects accounted for nearly 60% of the total genetic variance in the *E. grandis* x *E. urophylla* hybrid factorial of their study in Zululand. Bouvet et al. (2009) reported an average $\hat{\sigma}_D^2/\hat{\sigma}_A^2$ ratio of 1.2 for a relatively large *E. grandis* x *E. urophylla* hybrid seedling population (684 families). One explanation of the high dominance variance could be due to the nature of dominance variance. The dominance effect between alleles and their frequency will determine the magnitude of the dominance variance (Lynch and Walsh 1998). Dominance variance exceeds additive variance in the case of overdominance and/or in the case of total dominance when frequencies of alleles are different when a model of one locus and two alleles are used (Lynch and Walsh 1998). Although the case of overdominance must be considered with caution (Birchle et al. 2006), it may explain some of the relatively high dominance variance present in perennial plants such as *Eucalyptus* (Bouvet et al. 2009). Nevertheless, these results indicate that a breeding strategy to capture non-additive genetic effects in the hybrid will be the most appropriate strategy to follow. Three strategies that could potentially exploit non-additive variance are RRS (Comstock et al. 1949), reciprocal recurrent selection with forward selection (RRS-SF; Nikles 1992) and the development and crossing of inbred lines. The implications of these strategies for tree improvement have been discussed by various authors

(Dungey et al. 2000; Hettasch et al. 2005; Nikles 1992; Shelbourne 2000; Vigneron 1991). Kerr et al. (2004) did a simulation study comparing RRS, RRS-SF, recurrent selection for general combining ability (RS-GCA; Jenkins 1940) and the hybrid swarm strategy over five cycles of breeding. Results from this study suggest that the RRS-SF strategy yielded the highest genetic gains per year in cases where non-additive variance is higher than additive variance and when the pure-hybrid correlations are negative or close to zero. However, the latter was not cost efficient at all.

Another important factor to consider when developing an interspecific hybrid breeding strategy is the weak correlations that were detected between *E. grandis* x *E. urophylla* hybrid seedling ortets and their ramets in our study. One possible reason for the weak correlation is the degree of environmental influence that was present in the *E. grandis* x *E. urophylla* hybrid seedling population. The H_t^2 was estimated as 0.37 and the h_t^2 as 0.12 in the seedling population. This is an indication that majority of the variance in the *E. grandis* x *E. urophylla* hybrid seedling population was explained by environmental noise and could have an impact on the ortet-ramet phenotypic correlation. Although we did investigate the correlation between family and individual breeding values of the *E. grandis* x *E. urophylla* hybrid seedling ortets and the clonal BLUP estimates of their ramets, a more detailed study is necessary to investigate the genetic correlation between eucalypts hybrid seedling ortets and their ramets.

Another fundamental difference between seedlings and clones that could potentially influence the correlation between ortet and ramets, is the structure of their root system (Hartmann et al. 1990). Sasse and Sands (1997) reported that *E. globulus* seedlings had strongly gravitropic tap-roots, with two types of primary roots from which secondary roots emerged. Clones had no tap roots, but it had adventitious roots that were formed during propagation. Grossnickle and Russell (1990) found that cuttings of *Chamaecyparis nootkatensis* produced less new root area than seedlings over 21 days. Fuller and Little (2007) also reported that *E. grandis* seedlings had significantly longer roots than micro-cuttings, as well as a better distribution around the plug. However, no significance in growth was reported in their study. Gaspar et al. (2005) also reported that there was no significant difference between *E. globulus* cuttings and seedlings for growth and wood density. Sasse and Sands (1996) conducted a study to test the responses of *E. globulus* cuttings and seedlings to water stress and reported that the seedlings had greater water use than cuttings in the water stress treatments. However, in all these studies cuttings were produced from different individuals with a similar genetic makeup than

the seedlings of a pure species population. No direct comparison of ortets and their ramets have been reported on. Other effects associated with cloning such as rooting ability of different individuals and C effects could also contribute to the difference in growth performance between *E. grandis* x *E. urophylla* seedling ortets and their ramets. C effects are related to non-genetic sources of covariance between ramets of the same clone and may be due to factors such as the age or the environment of the original ortet (Costa e Silva et al. 2004). When C effects are present, it may inflate estimates of between-clone variances (Libby and Jund 1962; Burdon and Shelbourne 1974), and may upwardly bias epistatic genetic variance estimates (Costa e Silva et al. 2004). Inequalities among propagules within clones due to ontogenic factors such as cutting position on the ortet or morphological factors such as cutting size may also arise with cloning and could affect growth performance (Costa e Silva et al. 2004). Nevertheless, if the correlation between the selected *E. grandis* x *E. urophylla* seedling ortet and their ramets are weak as suggested in our study, then the feasibility of selection at seedling level needs to be investigated.

CONCLUSIONS

The study was set out to review the current *E. grandis* and *E. urophylla* hybrid breeding strategy used in South Africa and has identified key areas to investigate.

Information on genetic parameters of *E. grandis* x *E. urophylla* hybrid seedling and clonal populations needed quantification. The pure-hybrid and ortet-ramet correlation values are essential to develop a suitable hybrid breeding strategy and were explored in this study. The general theoretical literature on this subject and specifically in the context of *E. grandis* x *E. urophylla* hybrid clonal populations is inconclusive on several vital questions within the hybrid breeding discourse. The study sought to answer some of these questions.

Results from our study indicated that non-additive variance plays a major role in determining the growth performance of *E. grandis* x *E. urophylla* hybrid seedlings and clones. Due to the pre-eminence of non-additive variance, the pure-hybrid correlations were weak, especially for clonal populations. It would therefore seem that GCA or individual breeding values are not good predictors of GHA for growth performance in the observed populations. Results from this study also indicated a weak ortet-ramet correlation for *E. grandis* x *E. urophylla* hybrids. This suggests that the current strategy to first screen *E. grandis* x *E. urophylla* seedlings in progeny trials should be revisited.

Although our study has offered an evaluative perspective on *Eucalyptus* hybrid breeding, it encountered a number of limitations, which need to be considered.

Firstly, all the *E. grandis* and *E. urophylla* parents used for interspecific crossing were selected on their mature age phenotypes for growth and tree form. This selection process might explain the lack of additive variance present in the observed *E. grandis* x *E. urophylla* hybrid populations.

Secondly, in view of the selected nature of *E. grandis* x *E. urophylla* ortets and the limited numbers of individuals per family, results on the *E. grandis* x *E. urophylla* clonal populations must be interpreted cautiously. Due to this limitation, the family effect of clonal populations was insignificant, and ortet-ramet correlations were restricted to phenotypic correlations.

The scale of this debate is therefore extensive and to develop a suitable and cost effective eucalypt hybrid breeding strategy, there is a need for more case studies to allow further assessments of this subject.

In spite of the limitations of this study, it has attained its primary objective namely: to review the current hybrid breeding strategy. From the results of the study, the overall recommendation is to adopt a hybrid breeding strategy that captures non-additive genetic

effects in combination with a strategy that minimises the testing time of *E. grandis* x *E. urophylla* hybrid material as seedlings.

ACKNOWLEDGEMENTS

The authors thank the Mondi Research Team for assistance with trial establishment and measurements. They also thank the Mondi management team for making the data available and for constant report. Funding was provided by Mondi training department.

REFERENCES

- Birchle JA, Yao H, Chudalayandi S. 2006. Unraveling the genetic basis of hybrid vigor. *Proceedings of National Academy of Science USA* 103: 12957-12958.
- Bison O, Ramalho MAP, Rezende GDSP, Aguiar AM, Deresende MDV. 2006. Comparison between open pollinated progenies and hybrid performance in *Eucalyptus grandis* and *Eucalyptus urophylla*. *Silvae Genetica* 55: 192-196.
- Bouvet JM, Saya A, Vigneron PH. 2009. Trends in additive, dominance and environmental effects with age for growth traits in *Eucalyptus* hybrid populations. *Euphytica* 165: 35-54.
- Burdon RD, Shelbourne CJA. 1974. The use of vegetative propagules for obtaining genetic and Physiological information. *New Zealand Journal of Forestry Science* 4: 418-425.
- Burdon RD. 1977. Genetic correlation as a concept for studying genotype-environment interaction in forest tree breeding. *Silvae Genetica* 26: 168-175.
- Comstock R, Robinson H, Harvey P. 1949. A breeding procedure design to make maximum use of both general and specific combining ability. *Agon Journal* 41: 360-367.
- Costa e Silva J, Borralho NMG, Potts BM. 2004. Additive and non-additive genetic parameters from clonally replicated and seedling progenies of *Eucalyptus globulus*. *Theoretical and Applied genetics* 108(6): 1113-1119.
- De Assis TF. 2000. Production and use of *Eucalyptus* hybrids for industrial purposes. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 63-74.

-
- Denison NP, Kietzka JE. 1992. The use and importance of hybrid intensive forestry in South Africa. In: Lambeth C, Devorak W (eds), *Proceedings of the IUFRO Conference: Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species, October 1992, Cali, Colombia*. pp 348-358.
- Dickerson GE. 1969. Techniques for research in quantitative animal genetics. In: *Techniques and procedures in animal science research*. Albany, New York: American Society of Animal Science. pp 36-79.
- Dungey HS, Kerr JR, Dieters MJ. 2000. A simulation of the comparative gains from three different hybrid breeding schemes (II) Results. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 351-356.
- Eisen E, Saxton A. 1983. Genotype x environment interactions and genetic correlations involving two environment factors. *Theoretical and Applied Genetics* 67: 75-86.
- Falconer DS. 1981. *Introduction to quantitative genetics*. United Kingdom: Longman Scientific & Technical.
- Fuller GM, Little KM. 2007. Fourteen month growth responses of *Eucalyptus grandis* micro-cuttings and seedlings and their interaction with weeding in Zululand, South Africa. *ICFR Bulletin* 17: 1-25.
- Gaspar MJ, Borralho N, Lopes Gomes A. 2005. Comparison between field performance of cuttings and seedlings of *Eucalyptus globulus*. *Annals of Forest Science* 62: 837-841.
- Grossnickle SC, Russell JH. 1990. Water movement in yellow-cedar seedlings and rooted cuttings: Comparison of whole plant and root system pressurization methods. *Tree Physiology* 7: 57-68.
- Hartmann HT, Kester DE, Davies FT. 1990. *Plant Propagation Principles and Practices* (5th edn). Englewood Cliffs: Prentice-Hall.
- Hettasch MH, Lunt KA, Pierce BT, Snedden CL, Steyn DJ, Venter HM, Verryn SD. 2005. *Tree Breeding Course Manual*. Pretoria: Natural Resources and Environment, CSIR.
- Hill WG. 1984. On selection among groups with heterogeneous variance. *Animal Production* 39: 473-477.

-
- Hodge GR, Dvorak WS. 2012. Growth potential and genetic parameters of four Mesoamerican pines planted in the Southern Hemisphere. *Southern Forests* 74(1): 27-49.
- Ikemori YK. 1984. The new eucalypt forest. In: *Proceedings of the Wallenberg Foundation Symposium, Falun, Sweden*. The Marcus Wallenberg Foundation. pp 16-20.
- Jenkins MT. 1940. The segregation of genes affecting yield of grain in maize. *Journal of the American Society of Agronomy* 32: 55-63.
- Kerr RJ, Dieter MJ, Tier B. 2004. Simulation of the comparative gains from four different hybrid tree breeding strategies. *Canadian Journal of Forests Research* 34: 209-220.
- Kha LD, Cuong NV. 2000. Research on hybridisation of some *Eucalyptus* species in Vietnam. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 139-146.
- Libby WJ, Jund E. 1962. Variance associated with cloning. *Heredity* 17: 533-540.
- Lynch M, Walsh B. 1998. *Genetics and analysis of quantitative traits*. Sunderland: Sinauer Associates.
- Max TA, Burkhart HE. 1976. Segmented Polynomial Regression Applied to Taper Equations. *Forest Science*. Vol. 2. pp 283-289.
- Nikles DG. 1992. Hybrids of forest trees: the bases of hybrid superiority and a discussion of breeding methods. In: Lambeth C, Devorak W (eds), *Proceedings of the IUFRO Conference: Resolving tropical forest resource concerns through tree improvement, gene conservation and domestication of new species, October 1992, Raleigh, USA*. North Carolina: North Carolina State University. pp 333-347.
- Potts BM, Dungey HS. 2004. Interspecific hybridization of *Eucllyptus*: key issues for breeders and geneticist. *New Forests* 27: 115-138.
- Potts BM, Volker PW, Tilyard PA, Joyce K. 2000. The genetics of hybridisation in temperate *Eucalyptus*. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 200-2011.
- Retief ECL, Stanger TK. 2009. Genetic parameters of pure and hybrid populations of *Eucalyptus grandis* and *E. urophylla* and implications for hybrid breeding strategy. *Southern Forests* 71(2): 133-140.

-
- Rezende GDSP, de Resende MDV. 2000. Dominance effects in *Eucalyptus grandis*, *Eucalyptus urophylla* and Hybrids. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 93-100
- SAS Institute. 2002. *SAS 9.1.3 help and documentation*. Cary: SAS Institute.
- Sasse J, Sands R. 1996. Comparative responses of cuttings and seedlings of *Eucalyptus globulus* to water stress. *Tree Physiology* 16: 287-294.
- Sasse J, Sands R. 1997. Configuration and development of root systems of cuttings and seedlings of *Eucalyptus globulus*. *New Forests* 14: 85-105.
- Shelbourne CJA. 2000. Some insights on hybrids in forest tree improvement. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 53-62.
- Van den Berg GJ, Verryn SD, Chirwa PW, van Deventer F. 2016a. Genetic parameters of measures of growth in *Eucalyptus urophylla* in South Africa. *Southern Forests: a Journal of Forest Science* (DOI: 10.2989/20702620.2016.1162616).
- Van den Berg GJ, Verryn SD, Chirwa PW, van Deventer F. *In press*. Genetic parameters and genotypy by environment interaction of *Eucalyptus grandis* populations used in intra-specific hybrid production in South Africa. *Southern Forests: a Journal of Forest Science*.
- Verryn SD. 2000. *Eucalyptus* hybrid breeding in South Africa. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland*. Australia: Department of Primary Industries. pp 191-199.
- Vigner P. 1991. Creation et amelioration de varieties hybrides d'*Eucalyptus* au Congo. In: Schönau APG (eds), *Proceedings of the IUFRO Symposium: Intensive forestry: the role of eucalypts, 2-6 September 1991, Durban, South Africa*. Pretoria: Southern African Institute of Forestry. pp 345-360.
- Vigner P, Bouvet JM, Gouma R, Saya A, Gion JM, Verhaegen D. 2000. Eucalypt hybrid breeding in Congo. In: Dungey H, Dieters M, Nikles D (eds), *Proceedings of the*

QFRI/CRC-SPF Symposium: Hybrid Breeding and Genetics of Forest Trees, 9-14 April 2000, Noosa, Queensland. Australia: Department of Primary Industries. pp 14-26.

Volker PW. 2002. Quantitative genetics of *Eucalyptus globulus*, *E. nitens* and their F₁ hybrid. PhD thesis, University of Tasmania, Australia.

Volker PW, Potts BM, Borralho NMG. 2008. Genetic parameters of intra- and interspecific hybrids of *Eucalyptus globulus* and *E. nitens*. *Tree Genomics and Genomes* 4: 445-460.

White TL, Adams WT, Neale DA. 2007. *Forest genetics*. Wallingford: CAB International.